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Research Note—

Survival of Exotic Newcastle Disease Virus in Commercial Poultry Environment Following Removal of Infected Chickens

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SUMMARY. During the first weeks of 2003, after exotic Newcastle disease (END) was confirmed in commercial layer flocks in Southern California, it became apparent that the virus survival information in the literature varied widely and was difficult to extrapolate to current local conditions. The END Task Force used the information available in the literature and the recommendations of research scientists to establish protocols for safely handling manure from infected and depopulated premises. In an attempt to gain more applicable knowledge in the management of contaminated poultry manure in the course of the END outbreak, this virus survival study was designed and implemented. Environmental drag swabs were tested for END virus from two of the early-infected commercial ranches that consisted of several houses following immediate removal of the infected flocks. A total of 293 samples, composed of 168 manure drag swab pools, 72 dropping board swab pools, and 38 compost swab pools from 3 houses (ranch 1), and 180 manure belt scraper swab pools from ranch 2 were analyzed for ND virus isolation and characterization for 21 consecutive days postdepopulation. Thirteen manure drag swab pools (from houses 1 and 3) and two manure dropping board swab pools (from house 3) collected from ranch 1 were positive for END virus at 0, 1, 2, 3, 4, 7, 10, 12, and 16 days postdepopulation. No END virus was isolated after the 16th day following depopulation from any of the samples. All samples from ranch 2 were negative during the entire observation period.

RESUMEN. *Nota de Investigación*—Sobrevivencia del virus exótico de la enfermedad de Newcastle en el medio ambiente de las instalaciones avícolas comerciales después de la remoción de las aves infectadas.

Durante las primeras semanas del año 2003, después de la confirmación de la presencia del virus exótico de la enfermedad de Newcastle en lotes de ponedoras comerciales en el Sur de California, fue aparente que la información encontrada en la literatura sobre la supervivencia del virus variaba ampliamente, siendo difícil de extrapolar a las condiciones locales actuales. El grupo a cargo del manejo del virus exótico de la enfermedad de Newcastle empleó información disponible en la literatura y recomendaciones de investigadores para establecer protocolos adecuados de manejo de la cama proveniente de galpones infectados despoblados. Se diseñó e implementó un estudio de sobrevivencia del virus con el fin de obtener un mayor conocimiento práctico sobre el manejo de la cama contaminada de los galpones durante el curso de una epidemia del virus exótico de la enfermedad de Newcastle. Se evaluó la presencia del virus exótico de la enfermedad de Newcastle en hisopos de barrido del medio ambiente de dos de las granjas comerciales (cada una conformada por varios galpones) infectadas después de haber sido removidos los lotes afectados. Se aisló y caracterizó el virus de la enfermedad de Newcastle a partir de muestras de hisopos durante un período consecutivo de 21 días posteriores a la despoblación. En la granja 1, se analizaron 293 muestras compuestas de 168 muestras de hisopos de barrido de las camas de los galpones, 72 muestras de hisopos de bandejas con heces y 38 hisopos de materias

fecales convertidas en abono. En la granja 2, se analizaron 180 muestras de hisopos de raspado obtenidos en las correas de transporte de heces. En la granja 1 se detectó la presencia del virus exótico de la enfermedad de Newcastle a los 0, 1, 2, 3, 4, 7, 10, 12 y 16 días posteriores a la despoblación a partir de 13 hisopos de barrido de materia fecal transformada en abono (proveniente de los galpones 1 y 3) y dos hisopos obtenidos a partir de las bandejas con heces (provenientes del galpón 3). No se aisló el virus exótico de la enfermedad de Newcastle después de los 16 días posteriores a la despoblación en ninguna de las muestras. La totalidad de las muestras de la granja 2 fue negativa durante todo el período de observación.

Key words: manure drag swabs, exotic Newcastle disease

Abbreviations: CAF = chorioallantoic fluid; END = exotic Newcastle disease; HI = hemagglutination inhibition; NDV = Newcastle disease virus; PCR = polymerase chain reaction

Following discussions with the Exotic Newcastle Disease Technical Advisory Panel (Brugh *et al.*, pers. comm.) and a review of the literature (1,2,3,4,5,6,7), a decision was made to initiate a task force field study to gain more specific virus survival data in naturally infected poultry ranches. The variation in published Newcastle disease virus (NDV) survival times outside the host has been primarily associated with temperature, moisture, environment, and the medium in which the virus was tested (3,4). Asplin (1) showed that the virus could survive on skin and in bone marrow of plucked and eviscerated chicken carcasses when held at 1.1 C to 1.7 C for 98 to 134 days, respectively. When the carcasses were unplucked, the survival times increased to 160 and 196 days, respectively, at the same temperature. When the experiment was repeated at −15.5 C, the survival rate for both sites increased to more than 300 days, and it did not matter whether the carcass was plucked or unplucked. In another experiment (1), the NDV was tested on filter paper at 36.6 C and survived for 6 hr but inactivated after 12 hr; on egg shell the virus was active after 24 hr but inactivated after 44 hr; in sterile feces the virus was active after 72 hr but inactive at 90 hr; in fluid suspension at 1.1–1.7 C the virus was active after 203 days but inactive after 217 days; in fluid suspension at 0 C the virus was active after 161 days but inactive after 175 days; and when dried on glass and stored at 1.1–1.7 C and 0 C the virus was found to be active after 396 days. In an experimental trial of VVND virus survival in litter at 23–29 C after housing VVND-infected chickens and turkeys, the virus could not be detected after 10 and 14 days (2). In a study using the GB Texas strain of NDV, the survival of NDV in some of the substrates that might be utilized by the virus in natural transmission was evaluated. These included sterile and nonsterile soil, water, earthworms, and planaria (3). NDV-seeded sterile and nonsterile soils maintained at about 20 C and 100% relative humidity survived 22 days. The same samples at 0% and 15% relative humidity were inactivated at 8 and 15 days (3). In earthworms fed NDV, the virus could be recovered at 4 days when held at 21 C, and virus remained viable at 18 days when held at 12 C (3). Planaria fed NDV had viable virus at 9 days and 3 days when held at 20 C and 31 C, respectively (3). In experimentally contaminated lake water, NDV could be recovered from 11 to 19 days depending on pH, aeration, the presence of salts, and organic material (3).

Infective secretions and excretions from poultry infected with NDV are the main causes of contamination of buildings, equipment, and the environment. Various chemical and physical conditions affect the survival of the NDV outside the host. The survival of NDV in the environment plays an important part in disease perpetuation and spread (4). In poultry houses previously occupied by infected stock, NDV has survived no longer than 7 days during summer, 14 days during spring, and 30 days during winter (4). Others found, similarly, that NDV-contaminated and uncleaned pens were no longer infective for NDV after 6 to 14 days (4). Still others found virulent NDV survived 53 days in hen-house litter. (4).

Under experimental conditions with small amounts of test material in stoppered vials, NDV has been shown to survive for extended periods of time depending on the temperature at which it was held. For example, NDV survived on soil samples for 25 days at 37 C, 71 days at 20–30 C, and 172 days at −11 to 36 C, 235 days at 3–6 C and 538 days at −26 C. In the same study using chicken feces, NDV survived 56 days at 37 C, 94 days at 20–30 C, 172 days at −11 to 36 C, 538 days at 3–6 C, and 538 days at −26 C (6).

The dramatic variations in survival of NDV in experimental *vs.* natural conditions on poultry

ranches illustrates the point that sampling on ranches that are naturally affected may be helpful in establishing ND eradication policy. Another factor to consider is the variation in survival of NDV outside the host is the deviations in thermostability between different strains of NDV. In such an experiment, 24 different strains of NDV were evaluated for loss of infectivity when heated at 50 and 56 C for 60 min. With respect to infectivity, the thermostable and thermolabile strains were uniformly encountered among the 12 avirulent and 10 fully virulent strains studied. Strains of both heat-stable and heat-labile hemagglutinin were found in the avirulent group, whereas, hemagglutinin of all virulent strains were heat stable (5).

Additional information that was needed by the task force included the effect of composting on the survivability of NDV in the poultry manure. In a study on the effect of composting poultry carcasses on survival of two virulent poultry disease viruses, it was determined that highly pathogenic avian influenza and adenovirus of egg drop syndrome–76 had mostly been inactivated in 10 days and were entirely inactivated by 20 days of composting (7). In Southern California, the mid-winter lower temperatures, higher humidity and moisture, and the presence of organic material to protect the virus favored a longer survival time probability. However, even in winter, the Southern California weather conditions are milder than they are in many other poultry-raising areas of the United States.

The purpose of this study was to provide more applicable virus survival information for the establishment of more accurate manure management protocols for the conditions being addressed in the 2002–03 exotic Newcastle disease (END) outbreak. In addition to attempting to determine END virus survival time under the existing local conditions, this study was also an evaluation of the swabbing procedure itself as a tool for evaluation of END virus survival in the layer ranch environment.

MATERIALS AND METHODS

Ranch description. Samples were collected from two poultry ranches, each of which was equipped with different manure-handling systems. Ranch 1 had 10 open-sided ''California-style'' layer houses. Each of the houses had sidewalls with curtains, which could be manually raised or lowered. Each house was split into two adjoining sections with five rows of two-tiered cages, with dropping boards and a manure pit and with concrete floors. Three of the ten houses had 30-wk-old White Leghorn laying hens and were chosen for the study because the flocks were showing compatible clinical signs (diarrhea, respiratory distress, and mucoid discharge from the mouth), and laboratory confirmation of END was made. The time from the first reported clinical signs of disease until depopulation on ranch 1 was 15 days. The drag swab sampling of the houses began the day depopulation was completed. The Newcastle disease vaccine protocol for ranch 1 was as follows: Live vaccine was given at 19 days, 29 days, and 7 wk of age, and killed vaccine was given at 15 wk of age.

Ranch 2 had 10 high-rise enclosed houses, either with curtain-sided walls or solid walls with upper and lower floors. The lower floor was used as a manure pit or a lower level of cages. The two houses from ranch 2 that were chosen for the study had solid sidewalls and a high-volume positive-pressure air circulation system. The houses were stacked eight cages high with an automatic egg belt collection system and an automatic manure belt disposal system. The chickens housed in these two buildings were reported to have had the most recent clinical signs of END on this ranch; however, there were few noticeable clinical signs at the time of depopulation. The time from the first reported clinical signs of disease until depopulation on ranch 2 was 26 days. The drag swab sampling of the houses began the day depopulation was completed. The ages of the layers in the houses sampled were 61 and 74 wk. The Newcastle disease vaccine protocol for ranch 2 was as follows: Live vaccine was given at 19 days, 29 days, and 7 wk of age, and killed vaccine was given at 15 wk of age. The environment, including debris on the manure belts and manure belt scrapers, was very dry because of the ventilation system, and a thin layer of droppings on the belts was evident.

Temperature measurement. Temperatures of manure stacks and compost were recorded at the time of manure sampling using Reotemp A-36 thermometer (Reotemp Instrument Corporation, San Diego, CA) that had a 1.2-m-long sensor probe. The temperatures were recorded at a depth of 18 to 26 cm in order to reflect the internal temperature of the manure and compost pile. Temperatures were taken only from ranch 1, because there were stacks of manure present and they could be probed with the thermometer. Temperatures were not obtained from ranch 2, because there were no stacks of manure, except for a thin layer of manure that remained on the scrapers.

Manure swab samples. A set of four sterilized rayon fabric swabs (10 cm × 22 cm; Johnson and Johnson Nu Gauze, 10.2 cm × 10.2 cm, four-ply; Arlington, TX) were used for sampling randomly selected rows or sections. The swabs were premoistened with 6 ml of brain heart infusion broth (Difco Laboratories, Lenexa, KS), placed in sterile plastic bags, and kept frozen at −20 C until thawed immediately prior to use at the selected study ranches.

Table 1. Detection of END virus from infected commercial poultry premises using rayon fabric swabs following depopulation (ranch 1).

Days postdepopulation	House #	Positive/No. tested	Manure temp. range (C)
0	3	2/5	Not recorded
1	1	2/5	Not recorded
1	3	1/5	13.9–15.5
2	1	1/5	13.99–15.5
2	3	2/5	15.59–19.4
3	1	2/5	15.59–21.1
4	3	1/5	15.59–26.6
7	1	1/5	15.59–26.6
10	3[A]	¼	15.59–26.6
12	3[A]	1/6	109–15.5
13–14	1–3	0/14	8.89–15.5
16	1, 2, or 3	1/15	Not recorded

[A]Swabs taken from dropping board.

A handle device made of a 90-cm-long metal pipe or polyvinyl chloride plastic pipe with a metal clip or ring attached to one end for holding the swab was used for drag swab sampling of the manure. A single-use latex glove (Perry® x-am gloves, Ansell Perry, Inc., Massillon, OH) was worn during the collection procedure and was changed after collection of swabs from each row or section. The full length of the manure dropping board (30 m in length) was swabbed, and the surface of the manure pit under each cage row was sampled by dragging the swabs one half the length of the row, which was about 30 m in length.

Compost samples were obtained from compost piles by pushing a swab 5 to 7.5 cm deep into the manure compost at 11 and 12 days postdepopulation. Four swabs were taken and pooled in each sterile plastic bag. A total of 293 pooled swab samples were collected during the study period from ranch 1: manure drag swabs ($n = 168$), dropping boards swabs ($n = 72$), and compost pile swabs ($n = 38$). In ranch 2, all the eight scrapers (an approximate total area of 8 m^2) were swabbed manually, and most contained accumulation of organic material that came off the belts but stuck to the scrapers. Swab pool samples ($n = 180$) from the scrapers were obtained at one end of the manure belt, and these represent the only sample type that was analyzed from ranch 2. A sample of unit was made of four pools of scraper swabs, and the sampling was repeated daily for 21 days, as in ranch 1. The scraper was chosen because it represented the entire manure belt system.

Virus isolation. The moist drag swab samples were squeezed aseptically, and the fluid was placed in 15-ml sterile conical plastic tubes and centrifuged at 1500 × *g* for 10 min at 4 C. Two milliliters of the supernatant was filtered using a sterile 0.45-μm polyethersulfone syringe filter (Whatman, Inc., Clifton, NJ) and was added to 1.3 ml of an antibiotic mixture consisting of penicillin (10,000 units/ml; Cardinal Health, ELK Grove, CA); streptomycin sulfate (2000 μg/ml; Sigma, St. Louis, MO); gentamicin sulfate (1000 μg/ml; Sigma), kanamycin sulfate (650 μg/ml); and amphotericin B (20 μg/ml). Following incubation at room temperature for 1 hr, 0.2 ml was inoculated into three 9-day-old specific-pathogen-free chicken embryos via the chorioallantoic route. The inoculated eggs were incubated at 37 C and candled for up to 5 days to observe embryo mortality. Embryos that died within 24 hr were discarded. Embryos that were dead within more than 24 hr were chilled, the chorioallantoic fluid (CAF) was harvested for hemagglutination testing, and the positive hemagglutination CAF was further tested for hemagglutination inhibition (HI) using specific NDV antiserum (8) The positive HI samples were identified as avian paramyxovirus and were further characterized by real-time polymerase chain reaction (PCR) with direct sequence analysis of the amplicon for the fusion protein cleavage site (Hietala, pers. comm.).

RESULTS

The results for ranch 1 are summarized in Table 1. During the 21-day sampling period, APMV 1 was isolated from 7.2% (13/168) of the pooled manure drag swabs and 2.8% (2/72) of the dropping board swabs. Reverse transcription–PCR further characterized the APMV 1 isolate as END virus. END virus was isolated either from house 1 or house 3 on days 0, 1, 2, 3, 4, 7, 10, 12, and 16 postdepopulation (Table 1). All pooled drag swab samples from house 2 were negative, except one pool of drag swabs reported positive for day 16. All 38 compost swab samples were negative. However, all 180-postdepopulation pooled swab samples from ranch 2 were negative for END virus during the entire sampling time. The temperatures of manure taken during the swab sampling ranged from 8.8 to 26.6 C.

DISCUSSION

The number of positive virus isolations was higher at the start of sampling and decreased to a single positive pooled sample on days 4, 7, 10, 12, and 16 postdepopulation. END virus was recovered in a single pool of manure swabs on the 16th day postdepopulation on ranch 1, house 2; however, it was speculated that it was not as likely that the sample on day 16 was from house 2, and it was possible that samples from houses 1 or 3 could have

been the source of contamination. END virus was not isolated from any of the swab samples collected from ranch 2 following depopulation, even though the sampling began on the day the chickens were removed from the houses and continued daily for 21 days. There are a number of possible reasons for not recovering virus from ranch 2. First, the time from first clinical signs reported in the layers on the ranch until depopulation was completed and swab sampling began was 26 days. According to several reports in the literature, this would be long enough to have significant drops in virus activity under some natural conditions in poultry houses (2,6). Second, the fact that the flock was well vaccinated for Newcastle disease most likely contributed to the reduced virus shedding in the environment. Third, the houses were high-rise style, with a high volume-positive ventilation system, and the manure was noticeably dry on the surfaces and particularly on the manure belts and scrapers. Fourth, the sampling scheme in houses equipped with the automatic belt system was limited to the areas at the end of the manure belts, where a scraper was located that removed the manure from belts. It was evident that the manure was very dry on the belts and scrapers. After initial negative samples were found in several of the houses on ranch 1, three houses were selected that had chickens with active clinical signs of END. The sampling began in these houses on the day the chickens were removed, which was 15 days after first clinical signs appeared. The houses on ranch 1 were all California-style houses with plastic curtains that are raised for ventilation. The cages were stacked two deep, with dropping boards protecting the lower cages. The manure drops to the floor and piles in a cone as it accumulates under the cages. The manure that accumulated in the cones was very dry except for the surfaces that were noticeably moist for the first few days and then dried rapidly after the chickens were depopulated. The weather was relatively dry and temperatures were 15.5–26.6 C, except for a few days during the sampling period when it rained. Only two positive samples were found on dropping boards, probably because this sampling was not begun until the 10th day. The high percent recovery of END virus in houses 1 and 3 was likely related to the active clinical signs of END and shedding of the virus by sick birds in these houses and to the shorter interval (15 days) between initial clinical sign observation and complete depopulation. The temperature readings were no different at depths of 30 to 60 cm into the stacks of manure. It appeared that the manure was stable at the ambient temperature, with no heat being generated in the stacked cones of manure at the time of sampling.

No differences were observed in house 2 of ranch 1 that would explain why no NDV positive swab pools were found, other than the variables that have already been suggested in the case of ranch 2.

Compost windrows were sampled on ranch 1 because they were available and because questions were being raised about the success of the composting process in eliminating the END virus. The compost swab samples were all negative. This result supports those of previous studies (7) that the method of composting being used was effective in eliminating the END virus, even though the number of samples taken in this study was very limited and conclusions could not be made beyond the negative results.

The mid-winter lower temperatures, higher humidity and moisture, and the presence of organic material to protect the virus are consistent with the literature in favoring a longer NDV survival time. However, even in winter, the Southern California weather conditions are milder than they are in many other poultry-raising areas of the United States. This study was a result of a rare opportunity in that the field and laboratory resources were available and several egg ranches had recently been diagnosed with END and the owners were willing to cooperate in the study. The conclusions from this study are that the results are consistent with those of other investigators who attempted to determine the persistence of NDV in poultry environments. The idea of using drag swabs as the sampling procedure of choice was a result of considerable experience with environmental sampling on commercial egg-laying ranches to monitor for *Salmonella enteritidis*. Utilizing this experience, the *S. enteritidis* environmental monitoring procedure was modified as an END virus sampling protocol. This study supports that the described drag swab protocol is a useful tool in evaluating ND virus survival and perhaps could be replicated to monitor other viral agents in the poultry environment.

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